ANTIDIURETIC HORMONE AND URINARY EXCRETION OF PROSTAGLANDIN E_2 IN THE RAT

NOREEN F. ROSSI

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Antidiuretic Hormone

and

Urinary Excretion of Prostaglandin E_2 in the Rat

Noreen F. Rossi
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This thesis is dedicated to the memory of

my Grandfather

who taught me

to act justly, to speak humbly, to touch compassionately, to love tenderly

and

to hope eternally

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Noreen F. Rossi
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Abstract

In vitro evidence indicates that PGE_2 production in renal medullary interstitial cells is stimulated by ADH and the PGE_2 inhibits the effect of this hormone on water transport. However, factors modulating renal production and excretion of PGE_2 in states of altered water balance have not been well defined *in vivo*. Therefore, studies were designed to measure $U_{PGE_2}V$ following endogenous ADH, exogenous ADH without dehydration, and dehydration without ADH using normal Sprague-Dawley (N) and Brattleboro (DI) rats. U_{PGE_2} (Institut Pasteur). Urine was collected on ice because thin layer chromatography indicated that room temperature (24 hrs) resulted in conversion of 17% of PGE_2 to PGA_2. Stimulation of endogenous ADH by 18 hr dehydration in N rats (U max 2673 ± 132 mOsm/kg) increased $U_{PGE_2}V$ from 7.9 ± 0.7 to 11.2 ± 0.9 ng/12 hrs ($p<0.05$). Low dose vasopressin (600 mU/kg body wt/day) was given to DI rats for 7 days. By day one, $U_{PGE_2}V$ increased from 6.9 ± 3.6 to 29.3 ± 4.7 ng/24 hrs ($p<.005$), while U_{osm} increased from 99 ± 4 to 203 ± 41 mOsm/kg. $U_{PGE_2}V$ increased further on day 4 to 61.4 ± 9.2 ng/24 hrs ($p<.005$ compared to day 1) without further increase at day 7. The effect of dehydration in the absence of ADH was investigated in DI rats by collecting urine during the 4th through 16th hr of dehydration, while body wt decreased by 5.7 ± 0.6 (4th hr) to 15.5 ± 1.0% (16th hr). $U_{PGE_2}V$ increased ($p<.001$) from 7.9 ± 1.5 ng/12 hr at baseline to 23.3 ± 2.8 ng/12 hr during dehydration with U_{osm} increasing from 108 ± 6 to 502 ± 56 mOsm/kg. These studies demonstrate that in addition to ADH, dehydration alone is a potent stimulus to the renal productior and excretion of PGE_2, an effect which may further modulate that action of ADH on the collecting duct.

Introduction

The single most important factor influencing renal water metabolism is antidiuretic hormone. This peptide hormone is synthesized by cells in the supraoptic and paraventricular nuclei of the hypothalamus and stored in the posterior pituitary.[171] Release of antidiuretic hormone is controlled primarily by plasma osmolality.[158] For plasma osmolality greater than 280 mOsmoles per kilogram water there is a positive linear correlation between plasma antidiuretic hormone concentration and plasma osmolality.[46, 127, 128] The functional characteristics of the osmoreceptor in the anterior hypothalamus may be modified further by changes in circulating intravascular volume and pressure.[46, 127] Unlike the exquisite sensitivity of the osmoreceptor to variations in solute concentration, substantial changes in volume are required to alter the release of antidiuretic hormone.[46]

Circulating antidiuretic hormone acts on renal collecting ducts and late segments of the distal convoluted tubule. Increased water permeability in isolated collecting tubules occurs only when the hormone is on the peritubular side of the cells.[69, 70] Retrograde injection of vasopressin into the collecting system of rats produces no antidiuretic action.[138]

The basolateral plasma membranes of the collecting duct cells have a vasopressin specific receptor which is associated with an adenyl cyclase that catalyzes the formation of cyclic adenosine 3', 5'-monophosphate (cyclic AMP) from adenosine 5'-triphosphate.[25, 126]

Binding of vasopressin to the receptor activates the adenyl cyclase.[25] The changes in water permeability after vasopressin stimulation are attributed to the increase in concentration of cyclic AMP.[103] Exogenous cyclic AMP mimics the actions of antidiuretic hormone. Methylxanthine inhibition of cyclic AMP-phosphodiesterase, the enzyme which catalyzes the breakdown of cyclic AMP, results in accumulation of endogenous cyclic AMP in renal tissue after vasopressin stimulation and increases the hydro-osmotic effect of the hormone.[69, 70]

Dousa and colleagues have proposed that the permeability of the luminal membrane is associated with a specific membrane protein which is a substrate for cyclic AMP-dependent protein kinase. Phosphorylation of the protein leads to a change in membrane structure and increased water permeability.[42] Exposing rat renal medullary slices to vasopressin activates protein kinase.[76] This activation is proportional to the concentration of cyclic AMP.[41] The protein phosphatase for dephosphorylation is also present in renal medulla; however, the subcellular localization of both the specific protein substrate for the vasopressin activated protein kinase and the specific protein phosphatase has not been determined. A causal relationship between this system and the vasopressin-induced changes in water permeability remains to be established as well.[40, 134]

The cellular action of antidiuretic hormone requires the integrity of microtubules and microfilaments. Drugs like colchicine, vinblastine or podophyllotoxin which disrupt microtubules or prevent their assembly block vasopressin- or cyclic AMP-induced osmotic water flow without altering sodium transport in the toad urinary bladder.[153]

The antidiuretic action of vasopressin in vivo is also antagonized by these agents, but they had no effect on adenylate cyclase, cyclic AMP-phosphodiesterase, or cyclic AMP-dependent protein kinase from rat renal medulla.[39, 152] These findings suggest a role for microtubules in mediating the action of vasopressin on the distal nephron and also that their involvement occurs after the cyclic AMP effects.

Microfilaments are cytoplasmic organelles composed of protein with biochemical properties similar to muscle actin.[12] The microfilaments terminate close to or may be directly attached to cell membranes.[111] The antibiotic cytochalasin B which disrupts microfilaments reversibly inhibits the hydro-osmotic effects of both vasopressin and exogenous cyclic AMP besides blunting the vasopressin-stimulated increase in endogenous cyclic AMP.[37]

When toad urinary bladder is exposed to vasopressin the lateral intercellular spaces widen suggesting an intercellular pathway in transepithelial water flow.[86, 156] If the toad bladder is first exposed to cytochalasin B and then to vasopressin striking morphologic changes occur. The most pronounced changes include accumulation of giant vacuoles intracellularly, cell swelling, and disruption of microfilaments along the basolateral membranes.[37, 141] Since the intercellular tight junctions are impermeable to water[68] these studies suggest that microfilaments are involved in vasopressin-induced transcellular flux of water by altering properties of the basolateral plasma membranes.

Other drugs also interfere with the cellular action of antidiuretic hormone. Lithium impairs vasopressin-stimulated water

transport in the toad bladder *in vitro* and in rat and dog kidney *in vivo*.[136, 137] Thus, lithium produces a nephrogenic diabetes insipidus by impeding steps proximal and distal to cyclic AMP generation: both the vasopressin-stimulated adenylate cyclase and a cyclic AMP-dependent protein kinase are inhibited.[38]

Demeclocycline inhibits both vasopressin-induced and cyclic AMP-induced hydro-osmotic response of toad bladder.[152] Besides inhibiting vasopressin-stimulated adenylate cyclase and cyclic AMP protein kinase *in vitro*,[43] demeclocycline appears to cause nephrogenic diabetes insipidus at least in part by binding to a specific epithelial cell protein required for the action of antidiuretic hormone.[35, 41, 136]

The sulfonylurea chlorpropamide causes increased release of endogenous antidiuretic hormone,[115] and potentiation of the renal action of submaximal doses of vasopressin.[79, 112, 113] The drug has no effect on water flow in the absence of vasopressin[22] or in the presence of maximal levels of the hormone.[174] Paradoxically, chlorpropamide inhibits the effects of cyclic AMP on water transport.[79, 114]

Early studies of drug-induced disorders of antidiuretic hormone action did not adequately investigate the possible role of naturally occurring substances which could modulate the renal response to antidiuretic hormone *in vivo*. For example, only recently have the prostaglandins been implicated in the mechanism of chlorpropamide-induced enhancement of vasopressin action.[175] The discovery of high concentrations of prostaglandin E_2 and the enzymatic machinery for prostaglandin synthesis in renal medulla made it seem plausible that

prostaglandins have a function in modulating water excretion.

Credit for the original description of the class of fatty acids now known as prostaglandins belongs to von Euler.[160] It was not until twenty-five years later that Bergström and Sjövall succeeded in isolating these compounds[15, 16] and elucidating their structures.[17]

The prostaglandins are twenty-carbon fatty acid derivatives of prostanoic acid (Figure 1). All the prostaglandins possess a C-15 hydroxyl group and a C-13:C-14 trans double bond. The discrete classes -- E, F, A, B, C, and D -- are distinguished by the functional groups on the cyclopentane ring (Figure 2). The individual prostaglandins differ further in the degree of unsaturation of the side chains, designated by the subscripts 1, 2, or 3. The terms α or β refer to the configuration of the C-9 hydroxyl group, the α configuration being cis to the carboxyl function.

Although recent evidence suggests that prostaglandin A_2[90] and prostaglandin D_2[120] as well as the related compounds thromboxane A_2[145] and prostacyclin[165] are present in renal tissue, prostaglandin E_2 and prostaglandin $F_{2\alpha}$ have long been considered the principal renal prostaglandins.

The biosynthetic pathway for the renal prostaglandins is depicted in Figure 3. The fatty acid precursor 5, 8, 11, 14-eicosatetraenoic acid (arachidonic acid) is released from the cellular phospholipid stores by phospholipase A.[89] The conversion of arachidonic acid into prostaglandins is catalyzed by prostaglandin synthetase in the presence of molecular oxygen. The synthetase a microsomal enzyme complex,[7, 27] consists of a cyclo-oxygenase, a peroxidase, and

an endoperoxide isomerase or reductase.[107]

Briefly, arachidonic acid undergoes oxygenation and cyclization in the presence of cyclo-oxygenase to form the endoperoxide intermediate prostaglandin G_2. Prostaglandin G_2, in turn, is converted to a second endoperoxide prostaglandin H_2 via peroxidase. At this point, the biosynthetic pathway diverges to give prostacyclin, thromboxane A_2, prostaglandin D_2, prostaglandin $F_{2\alpha}$, or prostaglandin E_2. Specifically, prostaglandin E_2 is produced by the action of endoperoxide isomerase on prostaglandin H_2.[49]

There is some evidence that other tissues have the enzymatic capability of converting prostaglandin E_2 to prostaglandin A_2.[34, 132] No studies have convincingly demonstrated prostaglandin E_2 dehydrogenase in renal medullary tissue.[24, 90, 149] Whether the small amounts of prostaglandin A_2 found by some workers is due to non-enzymatic dehydration of prostaglandin E_2 to prostaglandin A_2[3] and that prostaglandin A_2 is indeed an artifact of the isolation procedures remains controversial.[91, 93]

Besides possessing the ability to synthesize prostaglandins, the kidney also has the capacity for their degradation. The enzymes responsible for prostaglandin E_2 metabolism in the kidney include 15-hydroxydehydrogenase (types I and II), Δ-13 reductase, and prostaglandin E_2-9-keto-reductase.

The 15-hydroxydehydrogenases are both cytoplasmic enzymes.[7] Type I uses NAD^+ as a cofactor and is located primarily in the renal cortex. Type II is more abundant in renal medulla where its specific activity is twice that in cortex. $NADP^+$ is the cofactor for the type

II dehydrogenase.[96]

Prostaglandin E_2 can be reversibly converted to prostaglandin $F_{2\alpha}$ by prostaglandin E_2-9-ketoreductase, an $NADP^+$-dependent soluble enzyme.[95] There is interspecies variability in the distribution of the prostaglandin E_2-9-keotreductase in the kidney[96, 143] as well as differences in the molecular properties of the enzymes from tissue to tissue.[143]

The rat kidney also has a prostaglandin $F_{2\alpha}$-9-hydroxydehydrogenase.[125] 13, 14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ can be dehydrogenated via this enzyme to 13, 14-dihydro-15-keto-prostaglandin E_2, a urinary metabolite of prostaglandin E_2. Hence, measurement of urinary metabolites of prostaglandin E_2 may not be a reliable index of prostaglandin E_2 metabolism.

Although isolated reports suggest that prostaglandin E_2 infused into the carotid artery inhibits water diuresis[159] and that intracisternal prostaglandin results in increased plasma antidiuretic hormone activity,[167] recent investigations have focused on the action of intrarenal prostaglandins to regulate vasopressin effects on water transport.

Very early work was performed on the toad urinary bladder. Basal water transport in the toad bladder is not affected by prostaglandin E_1.[101] However, prostaglandin E_1 uniformly antagonizes the permeability response of toad bladder to vasopressin and theophylline.[2, 56, 123] In these experiments prostaglandin E_1 does not influence the hydro-osmotic effect of exogenous cyclic AMP.[2, 123] Polyphoretin phosphate, a competitive antagonist of prostaglandins,

but not indomethacin counteracts the toad bladder response to vasopressin in the presence of exogenous prostaglandin E_1.[2, 124] In fact, in the absence of prostaglandins the competitive inhibitor exhibits a slight but measurable enhancement of vasopressin effects on water permeability.[124] Inhibition of endogenous prostaglandin E_2 synthesis by indomethacin increases the osmotic water flow response to submaximal doses of vasopressin and cyclic AMP.[55]

Intravenous infusion of prostaglandin E_1 results in urinary excretion of the corresponding metabolites.[67, 74] Excretory clearance of intact prostaglandin from the circulation is negligible.[67] Frölich and co-workers showed that unilateral renal artery infusion of the prostaglandin precursor sodium arachidonate or of angiotensin leads to an ipsilateral increase in urinary excretion of prostaglandin E as measured by competitive protein binding.[62]

Though previous investigators found essentially no prostaglandin E_2 activity in extracts of rabbit renal cortex,[87] Larsson and Anggard detected measurable amounts of prostaglandin E_2 in cortex. However, these investigators confirmed that prostaglandin E_2 biosynthesis is ten times higher in the medullary region of the kidney compared to the cortex.[92] More recently, evidence from stop-flow studies in dogs suggests that the site of entry of prostaglandin E into tubular fluid may be the loop of Henle.[166] It remains unclear if the bioassay utilized by Crowshaw and Szlyk was sensitive enough to detect the minute quantities of cortical prostaglandin E_2.

In microdissected slices of rabbit papilla, 39 to 42 percent of prostaglandin synthetase activity is associated with cells of the

collecting duct, but substantial activity remains in papillary slices lacking these cells.[26] This finding is not in total agreement with previous histochemical data that localized prostaglandin synthetase exclusively to collecting ducts.[84, 85] By immunofluorescence techniques cyclo-oxygenase is found primarily in the collecting duct but also to a lesser extent in the interstitial cells of the renal medulla of the rat.[139] Both renomedullary interstitial cells[30] and collecting duct cells[29] possess peroxidase activity. Whether this peroxidase is indeed the one involved in prostaglandin biosynthesis remains to be proven conclusively.

Similar cytochemical studies have demonstrated 15-hydroxydehydrogenase in cells of the thick ascending limb and the distal convoluted tubule in the cortex.[119] In vitro prostaglandin E_1 is concentrated by renal cortical slices with a tissue to medium ratio of up to six to one. No such uptake is exhibited by medulla.[23] Thus, the prostaglandin E_2 found in the cortex may in fact have been synthesized in the medulla and transported via tubular fluid to the cortex where it could be concentrated and then metabolized by the 15-hydroxydehydrogenase.

In the red hamster prostaglandin E_1 does not inhibit renal adenyl cyclase activity directly but does antagonize the vasopressin-stimulated increase in the enzyme's activity.[109] Experiments with low concentrations of prostaglandin E_1 in rat renal medulla corroborate these findings.[11] The permeability response of isolated perfused collecting tubules to vasopressin decreased approximately fifty percent in the presence of prostaglandin E_1, whereas tubules exposed to

prostaglandin E_1 alone displayed an increased water reabsorption.[70]

There is evidence that prostaglandin E diminishes the permeability of the collecting duct *in vivo*, hence producing a rise in free water clearance.[108] Inhibition of endogenous prostaglandin synthesis potentiates the peripheral action of vasopressin in conscious dogs[50] and in anesthetized hypohysectomized dogs undergoing a water diuresis.[5] Prostaglandin inhibition may decrease medullary blood flow thereby enhancing the osmotic gradient for water reabsorption.[88] The *in vitro* potentiation of vasopressin by prostaglandin inhibition could be accounted for on this purely hemodynamic basis without altering the cellular action of vasopressin. However, indomethacin also potentiates the action of vasopressin in conscious rats in which renal hemodynamics did not change.[102] Also, in renal papillae snap frozen in liquid nitrogen for cyclic AMP assay, the vasopressin-stimulated increase in cyclic AMP was enhanced after treatment with indomethacin[18, 102] -- further evidence to support the hypothesis that prostaglandins interfere with the cellular action of vasopressin to generate cyclic AMP.

Infusion of prostaglandin E_1 uniformly increases urine flow and free water clearance and decreases urine osmolality while renal blood flow and solute excretion simultaneously increase.[18, 72, 102, 135, 151] In agreement with these findings, water restricted dogs have diminished free water reabsorption after prostaglandin E_1 infusion into the renal artery.[72] These effects require the presence of endogenous antidiuretic hormone.[21] Although an increase in the distal delivery of tubular fluid to the diluting segment of the nephron can account for

the increase in water excretion, this does not exclude the possibility that prostaglandin E also inhibits the action of antidiuretic hormone on a cellular level. This hypothesis is substantiated by experiments showing that prostaglandin E_1 administration results in a greater decrease in free water reabsorption and hypotonic urine than acetylcholine, a vasodilator, despite equivalent solute excretion in saline expanded dogs.[108] Recently, Stygles and Hood have proposed that prostaglandin E_2 antagonizes antidiuretic hormone by increasing intracellular free calcium ion which then results in disaggregation of the microtubular apparatus.[144]

Additional evidence that in situ prostaglandins play a role in the renal water transport mechanism is provided by experiments in which rabbit renomedullary interstitial cells grown in tissue culture synthesize prostaglandins.[117, 173] Using this model system, Zusman and Keiser subsequently demonstrated that prostaglandin E_2 biosynthesis is stimulated by the presence of vasopressin.[174] Both vasopressin and dDAVP (1-desamino-8-d-arginine vasopressin) increased rabbit urinary prostaglandin E_2 excretion in vivo.[99]

Prostaglandins may also be involved in the maintenance of the medullary solute gradient. Rat renal papillary sodium concentration nearly doubles while chloride ion concentration increases sixty percent thirty minutes after treatment with prostaglandin synthetase inhibitors. Medullary plasma flow remains constant.[66] Thus, prostaglandins may modulate net renal water transport by this mechanism as well.

Much work has been done to show that prostaglandins are in-

volved in regulating renal water transport via the peripheral action of antidiuretic hormone; however, some investigators maintain that many of the results can be explained on the basis of intrarenal hemodynamic changes produced by the prostaglandins.[72] In vivo studies have been particularly complicated by variations in renal blood flow and renal vascular resistance which, in turn, may influence net water transport. In many instances it is unclear whether these hemodynamic changes are indeed the mechanism whereby prostaglandins exert their effects or the experimental conditions induced the changes in blood flow. For example, anesthetized acutely operated animals show a distinct decrease in renal blood flow after inhibition of prostaglandin synthetase.[155] This decrease in renal blood flow can be blocked by prior treatment with sar^1, ala^8-angitensin II, an antagonist of angiotensin II.[130] In contrast, conscious dogs given indomethacin have no change in basal renal blood flow.[148] Thus stimulation of the animals can lead to release of angiotensin which in itself is known to increase renal prostaglandins.[1, 106] Norepinephrine has a similar effect.[63] It is thought that experimental manipulation can potentiate the inhibitory actions of the prostaglandin synthetase antagonists.[155]

Prostaglandin E infusions also affect the distribution of renal blood flow. Increased renal blood flow is seen with prostaglandin E_1, prostaglandin E_2, and arachidonic acid administration in dog[8, 31] and rat.[45] The magnitude of these vascular changes is more profound in the inner cortex than the outer cortex,[31] but other vasodilators also show a preference for augmenting inner cortical blood

flow.[142] It may be that prostaglandins mediate the effects of these vasodilators as well.[174] Conversely, with the inhibition of prostaglandin synthesis the attending decrease in renal blood flow is accompanied by redistribution of flow to outer cortical nephrons.[88] Solez and colleagues have measured a diminished medullary blood flow in rats after indomethacin treatment.[140]

Much of the information on renal prostaglandins and their role in renal physiology has been gained through in vitro studies, and other work has largely involved infusions of prostaglandin E_1 which is not found in the kidney. The role of endogenous renal prostaglandin E_2 in mammalian systems in vivo has only recently been examined. Efforts in this area have largely been hindered by methodological problems in purifying and measuring the minute quantities of prostaglandins in physiological fluids.

Numerous and diverse techniques have been developed for isolation and quantitation of prostaglandins: gas liquid chromatography-mass spectroscopy,[9] competitive protein binding,[61] isotope derivative methods,[28] bioassay,[78] and radioimmunoassay.[80] Although gas liquid chromatography-mass spectroscopy is the most specific and accurate method available, it requires expensive equipment and most importantly lacks the sensitivity for measuring picogram quantities of prostaglandins in physiological fluids.

Radioimmunoassay appears to be the most promising analytical tool at this time although there are several pitfalls. The sensitivity of radioimmunoassay is very high (to concentrations of five and ten picograms per milliliter); however, its specificity

may often fall short for certain applications. This has been a problem especially with regard to developing antisera to prostaglandins of the E series. Many investigators have attempted to prepare antibodies to the prostaglandin E's with variable success.[44, 80, 81, 82, 83, 131] Cross reactivity between prostaglandin E_1 and prostaglandin E_2[122] and between these prostaglandins and their metabolites[81] has been the major impediment and is the reason radioimmunoassay tends to overestimate the amount of prostaglandin E.

In addition, conjugation of prostaglandin E to the carrier protein via a reaction with carbodiimide results in significant quantities of prostaglandin B being formed; thus, the resultant antiserum possesses considerable affinity and cross reactivity with prostaglandin B.[98] Some investigators have circumvented this problem by quantitatively converting prostaglandin E to prostaglandin B in a controlled reaction and then assaying the mixture for prostaglandin B activity.[131]

The development of a highly specific anti-prostaglandin E_2 antiserum at the Institut Pasteur[44] enables picogram quantities of prostaglandin E_2 in urine to be measured accurately after treatment of the urine with amberlite[59] and silicic acid column chromatography.[81]

The objective of the present experiments was to establish a sensitive radioimmunoassay capable of measuring urinary prostaglandin E_2 excretion during water diuresis and antidiuresis in small rodents. The effects of temperature and time on the stability of prostaglandin E_2 in urine was examined to determine the ideal conditions for collection

and storage of urine prior to radioimmunoassay. Measurement of urinary prostaglandin E_2 was determined following physiological stimulation of endogenous vasopressin in the Sprague-Dawley rat. Serial changes in urine osmolality and urinary prostaglandin E_2 excretion were also investigated in the Brattleboro strain of rats with congenital diabetes insipidus given physiological doses of exogenous vasopressin. The excretion of prostaglandin E_2 during dehydration in the absence of antidiuretic hormone was also investigated. These experiments were designed to determine the relationship between urinary excretion of prostaglandin E_2 and the hydro-osmotic action of antidiuretic hormone.

Methods and Materials

Methods

Effects of storage temperature and time on the stability of prostaglandin E_2 in rat urine

The effects of storage temperature and time on the conversion of urinary prostaglandin E_2 to prostaglandin A_2 were determined in thin layer chromatographic studies. Adequate urine volume for all studies was obtained from a 24-hour collection in a 253 gm Brattleboro rat with free access to water and standard ground Purina rat chow.

The urine was partitioned into 1.0 ml fractions in 50 ml conical tubes. At time zero (t_0), approximately 30,000 cpm ^{3}H-prostaglandin E_2 in 10 µl ethanol were added to each sample and mixed thoroughly by vortex agitation. Samples were then assigned to one of three groups: room temperature ($22^o \pm 3^oC$), standard refrigerator temperature (4^oC), or freezer temperature (-20^oC).

After an interval of time (t_n) measured from t_0, varying from zero minutes to 14 days, each fraction was extracted with 8.0 ml ethyl acetate. Extraction of frozen urines was accomplished at the moment the samples had thawed completely. For these samples, t_n equaled the time elapsed from t_0 to initiation of the thawing process. The ethyl acetate extract was evaporated under nitrogen at room temperature and the residue was dissolved in 500 µl ethyl acetate: methanol, 1:1, v:v. The concentrated extract was spotted on silica

gel thin layer chromatography plates. As markers, duplicate standards containing 100 μg prostaglandin A_2, 200 μg prostaglandin E_2 and 100 μg prostaglandin $F_{2\alpha}$ in 300 μl ethanol were spotted on peripheral channels of each plate. Each 20 cm x 20 cm plate accomodated six samples.

To control for any ^{3}H-prostaglandin A_2 in the exogenous tracer that might interfere with the estimates of conversion of prostaglandin E_2 to prostaglandin A_2, a 10 μl sample of ^{3}H-prostaglandin E_2 in ethanol was plated directly (control #1). To demostrate that the method was capable of detecting conversion of prostaglandin E_2 to prostaglandin A_2, a second control (control #2) was prepared. A urine sample with ^{3}H-prostaglandin E_2 was titrated to pH 3 with 0.10 N hydrochloric acid prior to extraction and plating; this procedure is known to accelerate the conversion of prostaglandin E_2 to prostaglandin A_2.[3]

Each thin layer chromatography plate was developed twice in the same direction with chloroform:methanol:acetic acid:water, 90:6:1:0.75, v:v:v:v. The prostaglandin markers were sprayed with 10% (w/v) phosphomolybdic acid in ethanol and visualized by dry heat. Zones with relative Rf's corresponding to the markers for prostaglandin A_2, prostaglandin E_2, and prostaglandin $F_{2\alpha}$ were scraped and vacuum aspirated into 6-inch Pasteur pipettes fitted with pyrex wool. The remaining areas of gel were treated similarly. The silica gel was eluted into scintillation vials with 6.0 ml methanol and samples were taken to dryness under air. To each vial was added 10 ml Aquasol and the samples were counted for five minutes.

Effects of endogenous antidiuretic hormone on urine osmolality and urinary excretion of prostaglandin E_2 in the Sprague-Dawley rat

Animals employed in these experiments were male Sprague-Dawley rats weighing between 255 gm and 275 gm. Rats were caged in pairs and allowed Purina rat chow and water ad libitum until the beginning of the experiment.

At the onset of an experiment, seven rats were weighed and placed into individual clean metabolic cages. The animals were allowed up to 20 gm per day of laboratory prepared food (470 gm sucrose, 300 gm casein, 100 gm lard, 50 gm corn oil, 24.4 gm NaCl, 11.6 gm K_2HPO_4, and 50 gm vitamin-mineral mix) providing 8 milli-equivalents sodium per day. Free access to water was permitted. As prostaglandins are known to be involved in the mechanism of renin release,[58, 162, 163, 164] a controlled high sodium intake was utilized to maintain plasma renin at relatively steady low levels.

On the eighth day, while continuing the stipulated diet each cage was equipped with a clean clamped funnel to collect all voided urine under mineral oil for 12 hours. At the inception of the urine collection the bladder of each rat was emptied by external massage and this urine was discarded. The procedure was repeated at the termination of the collection period and this urine combined with the volume accumulated in the metabolic funnel. Fecal contamination was prevented by interposing a fine mesh wire screen between the rat and the urine. Throughout the 12-hour period the amassed urine was kept at $0^{o}C$ by immersing the funnels in ice-water baths replenished with ice every four to six hours as needed. After immediate deter-

mination of urine volumes, 7 ml aliquots of the specimens were frozen at -20°C for subsequent measurements of osmolality, creatinine, and prostaglandin E_2.

On the tenth day water was withdrawn from the rats. The animals were dehydrated for 30 hours. In order to obtain adequate urine during maximum stimulation of antidiuretic hormone secretion, urine was collected only between the 18th and 30th hours (12-hour collection). The collection procedure and handling of specimens were identical to that outlined above for the baseline determination.

Effects of exogenous vasopressin on urine osmolality and urinary prostaglandin E_2 excretion in the Brattleboro rat

Twelve 240 gm to 275 gm male Brattleboro rats which had previously been fed Purina rat chow pellets and water ad libitum were assigned to individual metabolic cages and allowed five days for acclimatization. During this time and the seven-day experimental period, the animals were offered water ad libitum and fed 15 gm ground Purina rat chow per day providing 1.5 milli-equivalents sodium per day.

On day one and each day thereafter for seven days, a subcutaneous injection of vasopressin tannate in oil, 600 mU per kilogram body weight was administered. Exactly 24 hours after the initial injection, the second injection was given. The rats were immediately induced to void by external bladder massage and a 24-hour urine collection was begun. The procedure for collection and storage of urine was identical to that for the 12-hour collection detailed in the preceding section.

Similar 24-hour collections were completed on the fourth and seventh days of vasopressin administration. All rats were weighed at the beginning of the experiment and on the seventh day.

Six hours after the final urine collection the animals were anesthetized with an intraperitoneal injection of Inactin, 100 mg per kilogram body weight. The inferior vena cava was surgically exposed and venous blood was collected into non-heparinized vacutainer tubes. The serum was stored at -20°C for subsequent creatinine determinations.

Effects of dehydration alone on urine osmolality and urinary prostaglandin E_2 excretion in the Brattleboro rat

Eleven male Brattleboro rats between 200 gm and 300 gm were caged in pairs and offered Purina rat chow pellets and water *ad libitum*. Five days prior to an experiment the rats were transferred to individual clean meatbolic cages and permitted to acclimate to the surroundings. During this time and the experimental period the animals were given 15 gm ground Purina rat chow per day and water *ad libitum* unless otherwise specified.

On the sixth day the rats were weighed and a baseline 12-hour urine collection was performed as described earlier in this text. Volume measurements were done on samples which were then frozen at -20°C.

The subsequent day the rats were weighed once again and water was withdrawn. After four hours dehydration, the animals' weights were checked and another 12-hour urine collection was executed. The rats continued to be deprived of water during the collection period.

At the end of 16 hours dehydration the rats were reweighed. Urine volumes were measured and the specimens were frozen at -20°C for later measurements of osmolality, creatinine and prostaglandin E_2.

The rats were anesthetized with Inactin as in the previous group and blood obtained for plasma creatinine.

Quantitation of urinary prostaglandin E_2 by radioimmunoassay

On the day of assay for prostaglandin E_2 the frozen urine specimens were thawed at room temperature. Then, 5.0 ml aliquots were immediately pipetted into individual 50 ml Nalgene screwcap centrifuge tubes. A 5.0 ml phosphosaline buffer blank and a control sample with 1.0 ng prostaglandin E_2 in 5.0 ml phosphosaline buffer were treated similarly.

For subsequent calculation of recovery, expressed as percent prostaglandin E, approximately 2000 cpm ^{3}H-prostaglandin E_1 in 50 µl ethanol were introduced into each sample and into duplicate scintillation vials for determination of total recovery. The tubes were vortexed. After each specimen had received 1.0 gm Amberlite CG-50, the tubes were placed at a 45 degree angle into a Dubnoff Mechnical shaker and agitated at the maximum setting (#9) for 20 minutes at room temperature. The tubes were then centrifuged at 5000 g for 10 minutes at 4°C, and the aqueous supernatant was aspirated and discarded. Elution of prostaglandins from the resin was accomplished by two washings (shaking 20 minutes at room temperature as above) with 5.0 ml methanol. Each extraction was followed by centrifugation as before. The organic phase was transferred to a 15 ml screwcap culture tube.

The combined methanol layers from the respective samples were dried under nitrogen at room temperature in an N-Evap for to a final volume of 1 ml to 3 ml.

After acidification with 25 µl 1.0 N hydrochloric acid, extraction with 8.0 ml ethyl acetate, and centrifugation at 1000 g for 10 minutes at 4°C, the top phase was pipetted into a clean 15 ml screwcap culture tube. The solvent was evaporated to dryness under nitrogen, and the residue was resuspended in 0.20 ml solvent IV (benzene:ethyl acetate:methanol, 60:40:10, v:v:v) and vortexed vigorously. Then 0.80 ml solvent I (benzene:ethyl acetate, 60:40, v:v) was added and the solution vortexed again. The prostaglandin solutions were capped and stored under nitrogen at -20°C overnight.

Isolation and purification of the prostaglandins was carried out by silicic acid column chromatography. One 16 cm x 1 cm Brock's mini-column with a piece of Whatman GF/A filter paper cut to fit the base with a #5 cork borer was set up for each separate sample. A slurry of 500 mg washed silicic acid in 5.0 ml solvent II (benzene: ethyl acetate:methanol, 60:40, v:v:v) was carefully transferred to the mini-columns by Pasteur pipette. When the solvent meniscus had reached the level of the silicic acid, the samples were applied to the columns. The fraction containing both prostaglandin A and prostaglandin B was eluted from the column with 4.0 ml solvent I and discarded. Next, 13.0 ml solvent II were utilized to elute the prostaglandin E; this fraction was collected in 20 ml screwcap vials. The solvent was evaporated to dryness under nitrogen, and the prostaglandin E redissolved in 1.0 ml ethanol.

To assess recovery an 0.10 ml aliquot of this ethanolic solution was pipetted into 10 ml Aquasol in a scintillation vial and counted for ten minutes. Yield was expressed as percent of total recovery.

The remainder of the prostaglandin E in ethanol was stored under nitrogen at -20°C until the time for quantitation of prostaglandin E_2 by radioimmunoassay the next day.

Duplicate 0.10 ml aliquots of the prostaglandin E solutions were pipetted into 10 mm x 75 mm RTU culture tubes. An occasional sample required 0.20 ml aliquots in order to obtain a quantity of prostaglandin E_2 within the range of the standard curve. A standard curve was pipetted from stock solutions (SS) of prostaglandin E_2 in ethanol: SS 1 contained 10.0 ng prostaglandin E_2 per 1.0 ml ethanol, SS 2 had 1.0 ng prostaglandin E_2 per 1.0 ml ethanol. Appropriate volumes of the stock solutions were pipetted in duplicate to obtain eight values (S1 to S8) ranging from 1 pg to 1 ng prostaglandin E_2 as shown in Table 1. Two tubes each for total counts (TC), non-specific binding (NSB), and initial binding (B_0) were also set up at this time.

After complete evaporation of the ethanol in a vacuum over at 15 mm Hg at room temperature, 0.10 ml phosphosaline buffer with 0.10% (w/v) gelatin was measured into each tube and vortexed. Ethanol in which the ^{3}H-prostaglandin E_2 was stored was evaporated under nitrogen and the ^{3}H-prostaglandin E_2 was brought up in a volume of phosphosaline buffer with gelatin so that 0.10 ml phosphosaline buffer with gelatin would deliver approximately 10,000 cpm to each

sample. All samples were again vortexed. Into all except the TC and NSB tubes was pipetted 0.10 ml anti-prostaglandin E_2 antiserum. The TC and NSB tubes received a second 0.10 ml phosphosaline buffer with gelatin without the antibody. After vortexing gently, the samples were allowed to stand at room temperature for exactly 10 minutes from the time of addition of the antiserum. Then, the tubes were transferred to an ice bath for 2 hours.

At the end of the reaction period, 1.0 ml dextran-coated charcoal solution was pipetted within 1 minute into all the samples except the TC tubes. Exactly 10 minutes later the samples were centrifuged at 2200 g for 10 minutes at $4^{o}C$. The supernatant was quantitatively decanted into individual scintillation vials already containing 15 ml Biofluor and 0.70 ml demineralized water. The vials were capped, vortexed and refrigerated until a clear homogeneous solution appeared at which time they were counted for 20 minutes.

All values for urinary prostaglandin E_2 were corrected for losses incurred through extraction and chromatography. In all the steps prior to radioimmunoassay prostaglandin E_1 and prostaglandin E_2 are indistinguishable; therefore, yield was estimated as percent of total recovery of added ^{3}H-prostaglandin E_1.

The affinity, specificity, and sensitivity parameters of the Pasteur anti-prostaglandin E_2 antiserum have been published.[44] Because of the weak cross reactivity of the antiserum with prostaglandin E_1 (2.7%),[44] no correction was made for the tracer amount of ^{3}H-prostaglandin E_1 added for recovery measurements.

Percent of trace binding was calculated as the fraction of

initial binding (B_0). Both values were corrected for nonspecific binding (NSB); that is,

$$\% \text{ trace binding} = \frac{x - NSB}{B_0 - NSB} \times 100$$

where x is the binding of the standard or test sample. For standards the resulting values were plotted on a log-logit scale. Percent trace binding for the test samples was determined from the above equation and the amount of prostaglandin E_2 read off the abscissa.

For determination of quenching, duplicate scintillation vials containing approximately 30,000 cpm ^{3}H-prostaglandin E_2 were counted with 15 ml Biofluor alone and after the introduction of 0.70 ml demineralized water and 1.3 ml phosphosaline buffer with gelatin. Percent quenching was calculated from the formula

$$\%Q = \frac{\text{cpm(Biofluor)} - \text{cpm(Biofluor:}H_2O\text{:PSBG)}}{\text{cpm(Biofluor)}} \times 100.$$

Background was assessed as cpm for a Biofluor:water:phosphosaline buffer with gelatin mixture without tritiated prostaglandin.

Materials

Sprague-Dawley rats were ordered from Charles River (Boston, MA). Brattleboro rats were from the colony of Dr. John N. Forrest, Jr.

Vasopressin tannate suspension in peanut oil for intramuscular injection was furnished by Park, Davis and Company (Detroit, MI). Inactin came from Hamburg, Germany.

Unlabelled prostaglandins for standards were generously supplied by Dr. John L. Pike of the Upjohn Company (Kalamazoo, MI). Tritiated prostaglandin E_1 {(5,6(n)-^{3}H} -prostaglandin E_1, Code TRK.426, Batch 12) was a product of the Amersham Searle Corporation (Atlington Heights, IL). Tritiated prostaglandin E_2, Lot 932 122) came from the New England Nuclear Company (Boston, MA). Both radioactive prostaglandins were purified by silicic acid column chromatography (see methods) within two weeks of use. All prostaglandins were stored in ethanol under nitrogen at -20°C.

Thin layer chromatography plates were prescored silica gel GF 250 μ thick from New England Nuclear.

In all cases ethanol was absolute ethyl alcohol purchased from U.S. Industrial Chemicals Company (New York, NY). Organic solvents were Mallinckrodt SpectrAR grade unless otherwise indicated and were used without further purification. Concentrated hydrochloric acid 37% (w/v) was obtained from Mallinckrodt, Incorporated (St. Louis, MO). All water was demineralized by passage through a D 5041 mixed resin filter of the Barnstead Sybron Corporation.

Sodium chloride (NaCl) and sodium hydroxide (NaOH) were reagent grade chemicals from Mallinckrodt. Sodium dihydrogen phosphate Monohydrate ($NaH_2PO_4.H_2O$), reagent grade, came from Fischer Scientific Company (Fair Lawn, NJ). Practical grade sodium azide (NaN_3) was provided by the Matheson, Coleman and Bell Manufacturing Chemists, Norwood, OH). Gelatin was obtained from Difco Laboratories (Detroit, MI).

Amberlite CG-50, 100 to 200 mesh, and silicic acid, 100 mesh, were products of Mallinckrodt. Prior to chromatography, silicic acid was washed three times with four volumes methanol to remove fines, dried in an oven at 40°C, and stored in a dessicator.

Phosphosaline buffer consisting of 0.10 M phosphate and 0.90% (w/v) NaCl with 0.10% (w/v) NaN_3 as preservative was brought up to pH 7.40 with NaOH. On day of radioimmunoassay, phosphosaline buffer with 0.10% (w/v) gelatin was prepared by dissolving 1 mg gelatin per 1 ml phosphosaline buffer over low heat and then cooling slowly to room temperature.

Dextran-coated charcoal was made by combining 300 mg Norit A (Fischer Scientific Company) with 100 ml phosphosaline buffer with gelatin at 0°C on a magnetic stirrer. When the charcoal had become nomogeneously dispersed in buffer, 30 mg Dextran T 70, Pharmacia (Uppsala, Sweden) was added. The mixture was stirred continuously and kept at 0°C for 30 minutes before and during use.

Anti-prostaglandin E_2 antiserum (immunogen:prostaglandin E_2-bovine serum albumin, animal: rabbit, Code 79 585 - Lot C) came in lyophylized form from Dr. F. Dray, Institut Pateur (Paris, France). The antiserum was stored at 4°C. At the time of assay the antibody was dissolved in 11.0 ml chilled phosphosaline buffer with gelatin and held at 0°C.

All centrifugations took place in a Sorvall RC-2 centrifuge manufactured by the Dupont Company (Norwalk, CT). Speeds are noted for the respective experiments.

Osmolalities were measured by freezing point depression on an osmomete from Advanced Instruments, Incorporated (Needham Heights, MA).

Evaporation under nitrogen was carried out on an N-Evap analytical evaporator by Organomation Associates, Incorporated (Shrewsbury, MA).

Assessment of radioactivity was performed in a refrigerated ($2^{o}C$) Packard Tri-Carb Liquid Scintillation Spectrometer Model 3320 (Downers Grove, IL). Scintillation fluid was Aquasol or Biofluor as specified from New England Nuclear. Times for counts are indicated for each determination.

Creatinine was measured employing a modification of the procedure of Folin and Wu on an Autoanalyzer, Technicon Corporation (Chauncey, NY).

Results

Effects of storage temperature and time on the stability of prostaglandin E_2 in rat urine

The thin layer chromatography experiments were designed to assess the optimal temperature for timed urine collections and storage of specimens in order to minimize loss of prostaglandin E_2 by conversion to prostaglandin A_2. The proportion of conversion was examined at three temperatures: room temperature ($22^{o} \pm 3^{o}C$), refrigerator temperature ($4^{o}C$), and freezer temperature ($-20^{o}C$). Since the partition coefficient for extraction of prostaglandins from aqueous to organic media is pH dependent, urine of identical pH (pH 6.3) from a single urine collection was used in all thin layer chromatography experiments (except Control #2). The relative amounts of tritiated prostaglandin in the prostaglandin E_2 and the prostaglandin A_2 zones was calculated as the percent of total recovered radioactivity for the given sample and are listed in Table 2.

After one hour, the radioactivity present in the prostaglandin A_2 zone of samples at any of the three temperatures did not differ substantially from the amount of radioactivity detected in the prostaglandin A_2 zone of Control #1 plated without processing through urine. The amounts of tracer found in the prostaglandin A_2 zone at room temperature, $4^{o}C$ and $-20^{o}C$ were 4%, 5% and 2% of total radioactivity, respectively. However, 4% of the tritiated prostaglandin in Control #1 was eluted from the prostaglandin A_2 zone.

From Figure 4 it can be appreciated that samples kept at $4^{o}C$ and

-20°C showed no change in the proportion of tracer converted to prostaglandin A_2 after a lapse of 24 hours, but at room temperature 17% of the added ^{3}H-prostaglandin E_2 was recovered from the prostaglandin A_2 zone. After 48 hours still no alteration in the distribution of tracer was discernible in the samples at 4°C and -20°C; however, following two weeks at 4°C this specimen contained 20% of the recovered labeled prostaglandin in the prostaglandin A_2 band. The percent of ^{3}H-prostaglandin E_2 recovered from the prostaglandin E_2 zone of this sample concomitantly decreased to 20% whereas in previous specimens the percent tracer in the prostaglandin E_2 band ranged from 65% to 86% (Table 2). Even after two weeks of storage at -20°C, the percent of tritiated prostaglandin in the prostaglandin A_2 zone (3%) did not differ from that in Control #1 (4%).

To evaluate the capability of thin layer chromatography to detect tracer amounts of prostaglandin A_2 produced from prostaglandin E_2 a positive control (Control #2) was prepared by adjusting the urine to pH 3 which is known to facilitate nonenzymatic conversion of prostaglandin E_2 to prostaglandin A_2.[3] After thus treating the samples, 23% (4°C) to 31% (RT) of the tritiated prostaglandin was eluted from the prostaglandin A_2 zone; the ^{3}H-prostaglandin E_2 remaining in the prostaglandin E_2 band decreased to 36% (4°C) and 41% (RT). For both these controls t_n equaled ten minutes.

To avoid artifactually inducing conversion of prostaglandin E_2 to prostaglandin A_2 in the test urines none of these urines were acidified before extraction and plating. Hence, recovery of total inoculated ^{3}H-prostaglandin E_2 after extraction, chromatography, and

elution varied over a wide range: 12.1% to 51.2%.

Effects of endogenous antidiuretic hormone on urine osmolality and urinary excretion of prostaglandin E_2 in the Sprague-Dawley rat

Dehydration of normal Sprague-Dawley rats was utilized to examine the effect of endogenous antidiuretic hormone secretion on urinary excretion of prostaglandin E_2. Average body weights of the rats at the inception of the experiment was 265 ± 3 gm. After eight days of sodium loading body weight averaged 306 ± 4 gm, an increase of 13.6 ± 0.9%.

As indicated in Table 3, urine flow during the 18th through 30th hours of dehydration was 6.1 ± 0.4 µl per minute which was a significant decrease ($p < 0.005$) from the baseline value, 13 ± 1.4 µl per minute. Predictably, urinary osmolality increased from 1268 ± 70 mOsm per kg at baseline to a maximum 2673 ± 132 mOsm per kg after dehydration ($p < 0.001$). Despite a marked reduction in urine flow, 12 hour excretion of prostaglandin E_2 increased from 7.9 ± 0.7 ng to 11.2 ± 0.9 ng ($p < 0.05$). Urinary creatinine excretion did not change significantly with dehydration.

Effects of exogenous vasopressin on urinary prostaglandin E_2 excretion in the Brattleboro rat

The effect of daily low dose exogenous vasopressin on renal water metabolism and excretion of prostaglandin E_2 was observed over seven days. Table 4 shows the serial changes in urine flow, urinary osmolality, and urinary excretion of prostaglandin E_2.

Over the course of vasopressin administration flow progressively

decreased while urinary osmolality concomitantly increased. There was an initial profound drop in flow from 73 ± 3 µl per minute to 43 ± 7 µl per minute after one day of vasopressin ($p < 0.005$). Further decreases in urine flow on days 4 and 7 were not significant when compared to the values on days 1 and 4. respectively.

Urine osmolality in the absence of antidiuretic hormone was 99 ± 8 mOsm per kg. Throughout the period of vasopressin administration there was continually statistically significant increments in urine osmolality reaching 860 ± 80 mOsm per kg on day 7.

Increases in urinary excretion of prostaglandin E_2 paralleled the changes in urine osmolality with a coefficient of correlation equaling 0.80 (see Figure 5). Twenty-four hour urinary output of prostaglandin E_2 after one day of vasopressin increased fourfold from 6.9 ± 3.6 ng at baseline to 29.3 ± 4.7 ng on day 1 ($p< 0.005$). UV_{PGE_2} again doubled by day 4 to 61.4 ± 9.2 ng ($p < 0.01$). There was no further change in urinary excretion of prostaglandin E_2 on day 7 despite the persistent rise in urine osmolality and fall in urine flow.

Creatinine clearance for nine rats was 1.16 ± 0.06 ml per minute.

Effect of dehydration alone on urine osmolality and urinary prostaglandin E_2 excretion in the Brattleboro rat

To examine if dehydration alone in the absence of antidiuretic hormone is sufficient to increase urinary excretion of prostaglandin E_2, Brattleboro rats were subjected to 16 hours of water deprivation.

Four hours after water had been withdrawn body weight had fallen by 5.7 0.6%. By the 16th hour of dehydration 15.5% 1.0% of initial body weight was lost. Average creatinine clearance for nine animals at the end of dehydration was 1.60 0.14 ml per minute.

Baseline urine osmolality (108 ± 6 mOsm per kg) was consistent with congenital diabetes insipidus; likewise, baseline urine flow was 96 ± 8 µl per minute. With dehydration urine osmolality rose to 502 ± 56 mOsm per kg ($p < 0.001$) and flow decreased to 17 ± 1.4 µl per minute ($p < 0.001$). Twelve hour urinary excretion of prostaglandin E_2 was 7.9 ± 1.5 ng at baseline. In the dehydrated animal UV_{PGE_2} increased to 23.3 ± 2.8 ng ($P < 0.001$).

Quantitation of urinary prostaglandin E_2 by radioimmunoassay

A typical standard curve for the radioimmunoassay of prostaglandin E_2 is depicted in Figure 6. Nonspecific binding was 4.6 ± 1.25 of trace binding. The phosphosaline buffer blank in each assay displaced less than 0.1% of bound radioactivity and thus was consistently outside the limits of the standard curve. The control with 1 ng prostaglandin E_2 in phosphosaline buffer gave 0.97 ± 0.10 ng by assay. Quenching was checked with each assay and remained stable at 15.9 ± 0.4%. Each value for urinary prostaglandin E_2 was individually corrected for loss during extraction and chromatographic procedures; average recovery was 38.6 ± 1.2%.

Discussion

Conservation of water by the mammalian kidney during periods of water deprivation or plasma hyperosmolality requires the presence of vasopressin and its effective action on the collecting duct in addition to the establishment and preservation of a hypertonic medullary interstitium.

The studies here have demonstrated that in saline-expanded Sprague-Dawley rats dehydration is a stimulus for renal production and excretion of prostaglandin E_2. In normal rats, dehydration is a potent stimulus for the secretion of antidiuretic hormone; however, other substances such as catecholamines and angiotensin II, which themselves influence renal synthesis of prostaglandin E_2,[1, 63, 106] are released as well. For example, acute hemorrhagic loss of 20% of blood volume in anesthesized dogs increased renal prostaglandin E_2; this increase was attributed to release of angiotensin II and increased renal nerve activity.[77, 146] During hemorrhagic shock, substances known to affect renal prostaglandin E_2 such as angiotensin,[1, 106] kinins,[174] and norepinephrine[63] are secreted in addition to antidiuretic hormone, thus making the change in prostaglandin E_2 excretion difficult to attribute to any single agent. Furthermore, we have demonstrated that marked dehydration in the absence of antidiuretic hormone increases urinary excretion of prostaglandin E_2. Although it is important to note that the degree of dehydration which accompanied the rise in renal prostaglandin E_2 is never achieved in rats with normal antidiuretic hormone secretory

function, it is nevertheless impossible to conclude unequivocally that *in vivo* stimulation of endogenous antidiuretic hormone would result in a quantifiable and significant rise in renal synthesis and urinary excretion of prostaglandin E_2.

However, there is convincing *in vitro* evidence that prostaglandin E_2 synthesis by renomedullary interstitial cells in tissue culture is stimulated by the presence of vasopressin.[174] In a recent abstract, Stein and Lifschitz reported that vasopressin administered to normal rabbits *in vivo* is associated with an increased urinary prostaglandin E_2 excretion.[99] Another preliminary study by Valtin and co-workers also found a rise in urinary excretion of prostaglandin E_2 after exogenous vasopressin was given for two days to Brattleboro rats.[48] The low doses of vasopressin administered in these *in vivo* studies still provided a higher level of the hormone than under normal physiological conditions of dehydration or states of plasma hyperosmolality. In light of these reports by other investigators, our data are consistent with, but do not conclusively prove, the hypothesis that endogenous antidiuretic hormone in itself may stimulate renal production of prostaglandin E_2.

If decreased intravascular volume were the sole factor involved in stimulating renal synthesis of prostaglandin E_2, Brattleboro rats, which are chronically in negative water balance and mildly volume contracted and have elevated plasma renin levels,[72] might be expected to show little change in urinary excretion of prostaglandin E_2 with exogenous vasopressin administration. However, our results indicate that these antidiuretic hormone-deficient rats have an acute rise in

urinary excretion of prostaglandin E_2 one day after low dose vasopressin. Significantly, prostaglandin E_2 excretion continues to rise for four days and is sustained at the new level while the rats' total body water is restored to normal. Frolich and colleagues have published preliminary data that shows this rise in renal production of prostaglandin E_2 is still evident after thirty days of exogenous vasopressin.[161]

Although when prostaglandin E_2 production is inhibited in conscious dogs there is no change in basal renal blood flow,[148] we may speculate that profound dehydration evokes an acute renal vasoconstrictor response which in turn may stimulate urinary prostaglandin E_2 excretion. This does not preclude the possibility that prostaglandin E_2 may act at the late distal convoluted tubule and collecting duct to regulate the cellular action of vasopressin. In fact, both actions of prostaglandin E_2 may be important for the optimal maintenance of water balance.

Since the available studies on urinary prostaglandin E_2 and vasopressin have appeared in abstract form,[48, 64, 99] the conditions for collection and storage of urine specimens prior to radioimmunoassay are not provided. The importance of collecting blood samples under strict conditions to avoid prostaglandin release from platelets has been stressed by Dray and co-workers.[44] We have systematically examined the conversion of prostaglandin E_2 to prostaglandin A_2 in rat urine and shown that collection at room temperature for 24 hours results in significant loss of prostaglandin E_2. Hence, the conditions for collection and storage of urine specimens for radioimmuno-

assay of prostaglandin E_2 must be strictly controlled. It is recommended that 24 hour urines be collected at ice-water temperature and subsequently stored at -20°C until assay.

In summary, both endogenous and exogenous vasopressin stimulates renal production and urinary excretion of prostaglandin E_2. However, renal prostaglandin E_2 produced in response to other stimuli such as dehydration may further modulate the cellular action of antidiuretic hormone on the collecting duct in states of altered water balance. The importance of collecting and storing urine for radioimmunoassay of prostaglandin E_2 under strictly controlled conditions which prevent chemical conversion of prostaglandin E_2 to prostaglandin A_2 has been demonstrated.

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Appendix

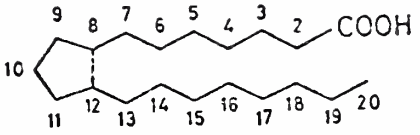

Figure 1. Prostanoic Acid

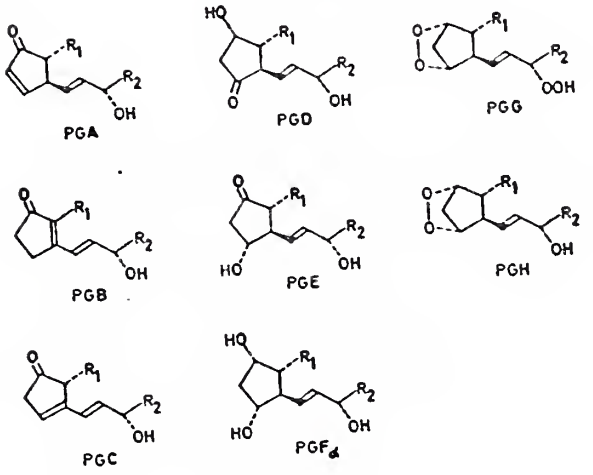

Figure 2. Structures of the Prostaglandins

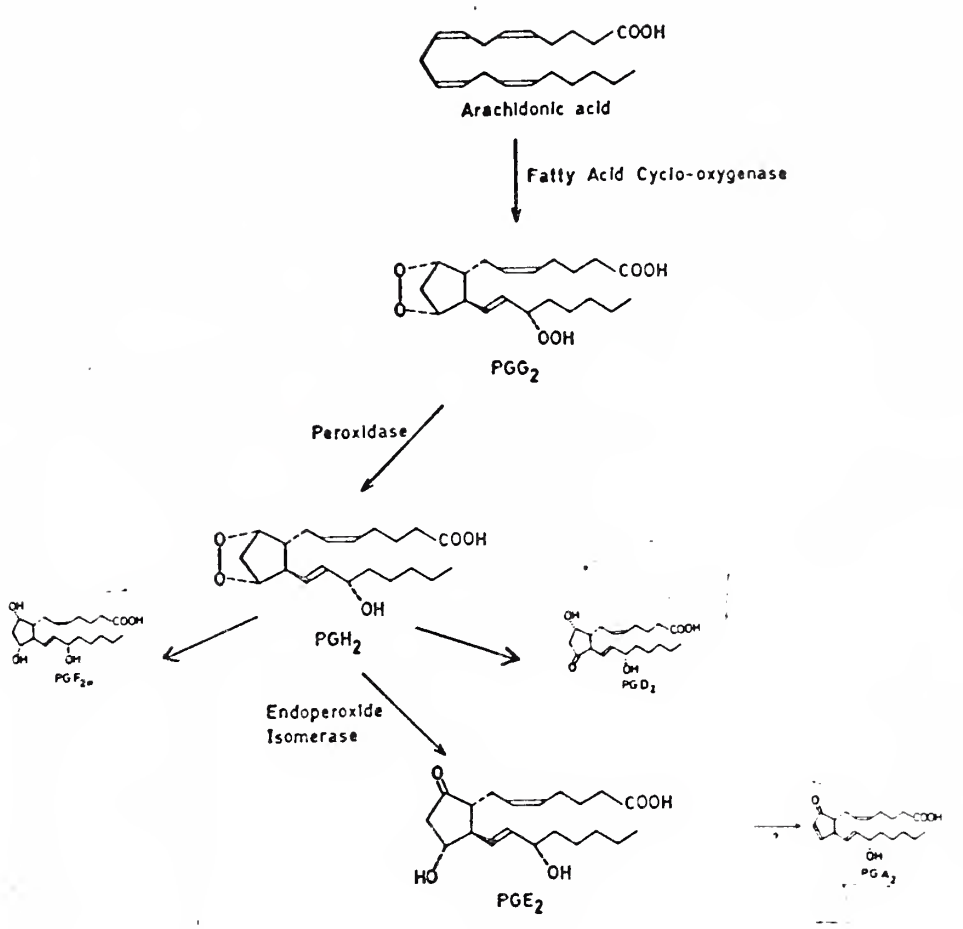

Figure 3. Pathways in the biosynthesis of PGE_2 from arachidonic acid.

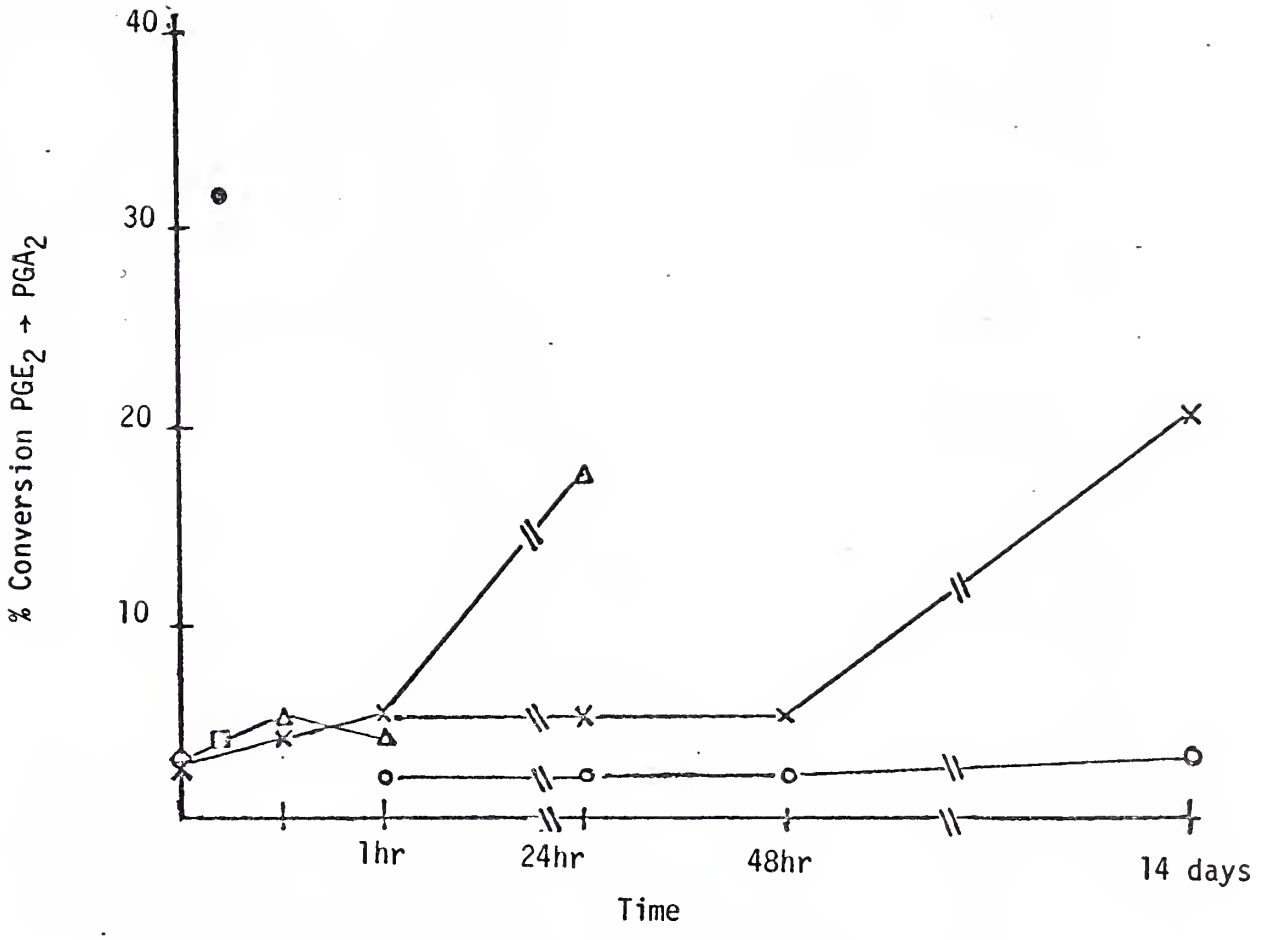

Figure 4. Effects of Storage Temperature and Time on the Stability of Prostaglandin E_2 in Rat Urine.

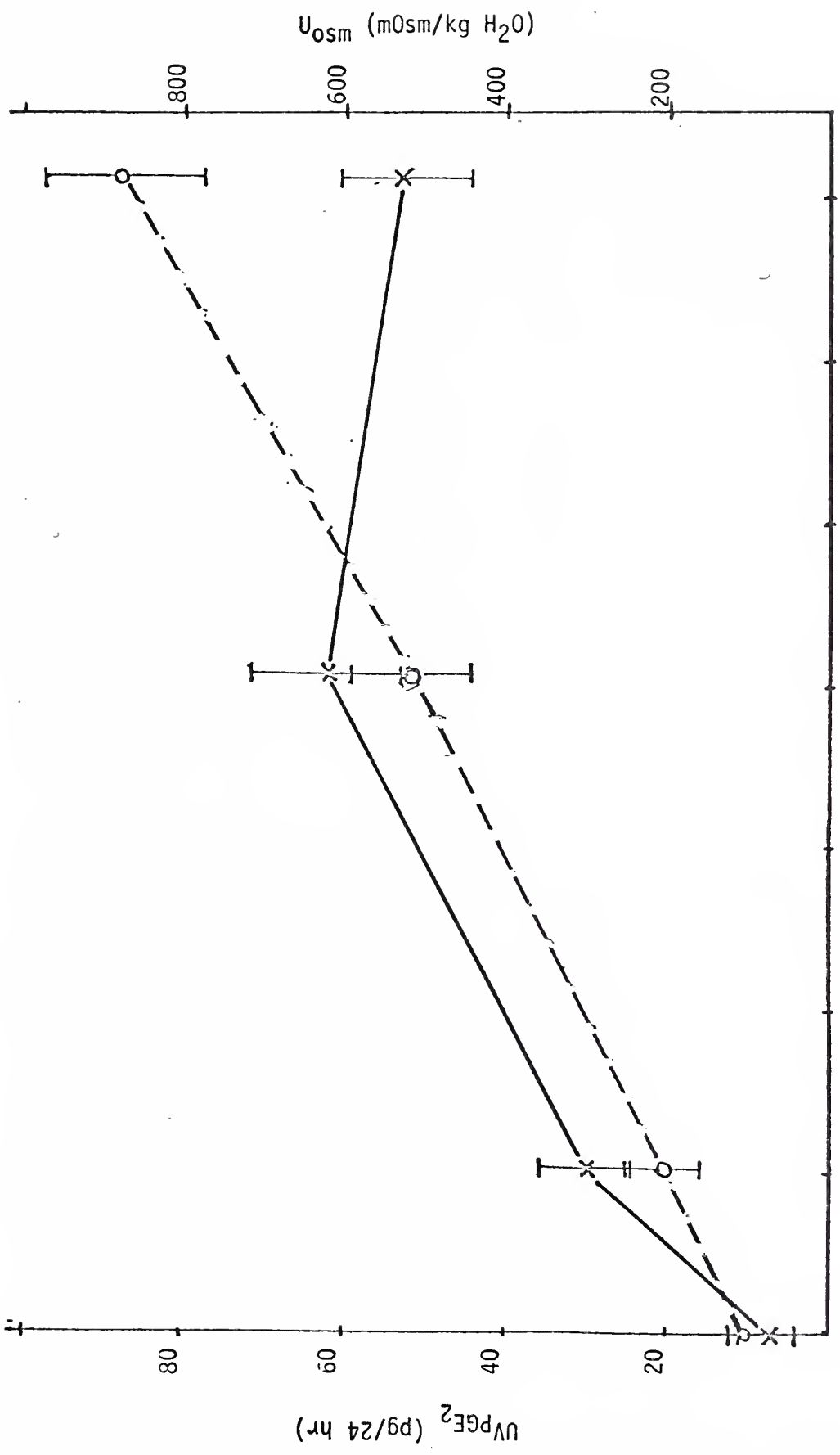

Figure 5. Serial Changes in U_{osm} and UV_{PGE_2} during 7 Days Exogenous Vasopressin

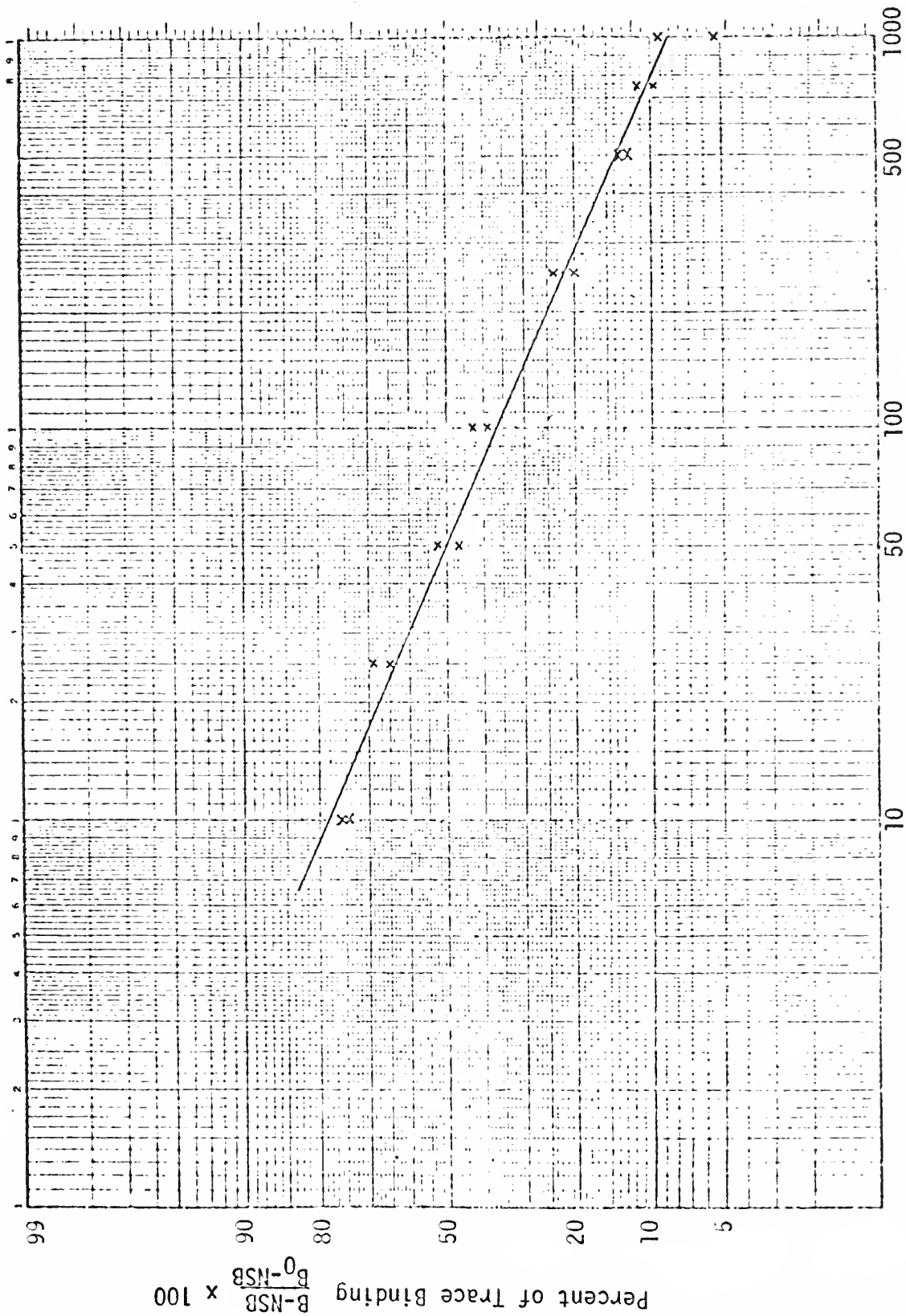

Figure 6. Typical Standard Curve for Prostaglandin E_2 Radioimmunoassay.

Table 1

Standard Curve for Radioimmunoassay

of Prostaglandin E_2

Standard No.	Quantity PGE_2 (pg)	Stock Solution No.	Volume (μl)
S 1	10	SS 1	10
S 2	25	SS 1	25
S 3	50	SS 1	50
S 4	100	SS 2	10
S 5	250	SS 2	25
S 6	500	SS 2	50
S 7	750	SS 2	75
S 8	1000	SS 2	100

Table 2

Effects of Storage Temperature and Time on the Stability of Prostaglandin E_2 in Rat Urine

	Room Temperature		4°C		-20°C	
t_n	% ^{3}H-PG in PGE_2 Zone	% ^{3}H-PG in PGA_2 Zone	% ^{3}H-PG in PGE_2 Zone	% ^{3}H-PG in PGA_2 Zone	% ^{3}H-PG in PGE_2 Zone	% ^{3}H-PG in PGA_2 Zone
0	0.77	0.03	0.77	0.02_5		
30 min	0.83	0.05	0.65	0.04		
60 min	0.85	0.04	0.86	0.05	0.83	0.02
24 hr	0.70	0.17	0.78	0.05	0.65	0.02
48 hr			0.82	0.20	0.75	0.02
14 days			0.20	0.20	0.71	0.03
Control #1 ^{3}H-PGE_2 in ethanol plated directly	0.81	0.04				
Control #2 Urine + ^{3}H-PGE_2 pH 3.0	0.41	0.31	0.36	0.23		

Key: t_n = time interval from t_0

Table 3

Effects of Endogenous Antidiuretic Hormone on Urine Osmolality and Urinary Excretion of Prostaglandin E_2 in the Sprague Dawley Rat. (n=7)

	Baseline	Post Dehydration	P
Flow (µl/min)	13±1.4	6.1±0.4	<0.005
Osmolality (mOsm/kg H_2O)	1268±70	2673±132	<0.001
U_{PGE_2} (pg/ml)	851±71	2628±298	<0.005
UV_{PGE_2} (ng/12 hr)	7.9±0.7	11.2±0.9	<0.05

All values given ± S.E. (standard error)

Table 4

Effects of Exogenous Vasopressin on Urine Osmolality and Urinary Prostaglandin E_2 Excretion in the Brattleboro Rat (n=12)

	Baseline	Day 1	Day 4	Day 7
Flow (µl/min)	73±3	43±7***	33±6^n	22±3^n
Osmolality (mOsm/kg H_2O)	99±8	203±41*	513±73***	860±80**
U_{PGE_2} (pg/ml)	59.5±28	664±143***	1466±171***	1765±193^n
UV_{PGE_2} (ng/24 hr)	6.9±3.6	29.3±4.7***	61.4±9.2**	51.8±7.4^n

Significance levels

* $p < 0.05$

** $p < 0.01$

*** $p < 0.005$

n < NS

Values for day 1 compared to baseline.

Values for day 4 compared to day 1.

Values for day 7 compared to day 4.

Table 5

Effect of Dehydration Alone on Urine Osmolality and Urinary Excretion of Prostaglandin E_2 in the Brattleboro Rat (n=11)

	Baseline	Post Dehydration	P
Flow (µl/min)	96±8	17±1.4	<0.001
Osmolality (mOsm/kg H_2O)	108±6	502±56	<0.001
U_{PGE_2} (pg/ml)	143±45	1935±250	<0.001
UV_{PGE_2} (ng/12 hr)	7.9±1.5	23.3±2.8	<0.001

All values given ± S.E. (standard error)

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